DEVELOPMENT OF A COLLAGEN-BASED SCAFFOLD FOR SEQUENTIAL DELIVERY OF ANTIMICROBIAL AGENTS AND PDGF GENES TO CHRONIC WOUNDS

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Chronic wounds are a global health burden affecting more than 5 million people in the United States alone. The complex wound microenvironment causes variable therapeutic outcomes following treatment with commercially available products. Wound infection is one of the major barriers in healing of wounds and localized delivery of antimicrobials is necessary for treatment. Furthermore, growth factors play a vital role in orchestrating the wound healing process through enhancement of cell proliferation, migration, and extracellular matrix remodeling. Accordingly, we have developed a collagen-based scaffold modified with combination of vancomycin-loaded liposomes and platelet derived growth factor (PDGF)-loaded DNA polyplexes. Both the liposomes and polyplexes were anchored to collagen using collagen mimetic peptides (CMPs). Our aim was to use CMP tethering to control the sequential release of vancomycin and PDGF polyplexes to immediately suppress infection and subsequently transfect wound bed fibroblasts with PDGF to assist the wound healing process. Vancomycin-loaded liposomes were prepared using dipalmitoylphosphatidylcholine (DPPC), cholesterol, and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)-2000] (DSPE-PEG). The liposomes were 160.7±2.1 nm in diameter based on dynamic light scattering (DLS) analyses, and the loading capacity of vancomycin was 51.5±0.7% in the liposomes. PDGF polyplexes (115.2±1.2 nm in diameter) were prepared by self-assembly of polyethyleneimine and PDGF plasmid DNA (N/P = 8) in 20 mM HEPES buffer (pH = 6.0), and successful PDGF gene loading was confirmed by agarose gel electrophoresis. Co-gels were prepared with collagen (4 mg/mL), fibrinogen (1.25 mg/mL), and thrombin (0.156 IU/mL) combinations that could successfully encapsulate both the vancomycin-loaded liposomes and PDGF polyplexes. Drug release studies confirmed that ~80% of the vancomycin was released during the 48 h study period, whereas PDGF polyplexes were retained longer (> 5 days) in the gel because their release requires collagen degradation mediated by matrix metalloproteinases present in the wound bed. The ability of the PDGF polyplexes to transfect fibroblasts was confirmed by *in vitro* cell transfection studies using green fluorescent protein (GFP) as a model gene. Furthermore, polyplex-mediated PDGF transfection was evaluated in fibroblasts cultured in an *in vitro* culture wound model, which showed that PDGF transfection enhanced migration rates of fibroblasts by ~2.4 fold as compared to controls in which culture wounds were allowed to heal in the absence of polyplexes. These results showcase the capacity for sequential delivery of vancomycin and PDGF gene *in vitro*, using collagen-based scaffolds, for potential applications in *in vivo* chronic wound treatments.